tissue surrounding the islets, which again reflects the state of activation of the macrophages. Finally, the vascular leakage was most prominent in the post-capillary venules, site of action of vasoactive monokines.

The microvascular changes observed early in the disease process may have important clinical implications. Indeed, it has been demonstrated that administration of serotonin and histamine inhibitors suppresses the development of diabetes in low-dose streptozotocin-treated mice [5]. These and other findings suggest the role of the pancreatic microvasculature, and possibly its interaction with mononuclear phagocytes, in the pathogenesis of diabetes, and we hope that our contribution may prove useful for further investigations in this field.

Yours sincerely,
M. E. De Paepe and E. Colle

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# Follow-up of anti-beta-lactoglobulin antibodies in children with Type 1 (insulin-dependent) diabetes mellitus

Dear Sir,
Recently Dahlquist et al. [1] reported that early exposure to cow's milk formula is related to an increased risk of early-onset Type 1 (insulin-dependent) diabetes mellitus. The authors found increased beta-lactoglobulin IgA antibodies and cow's milk protein IgA antibodies in children with early-onset Type 1 diabetes and suggest that early exposure to beta-lactoglobulin in genetically susceptible children might be one trigger in the autoimmune process leading to development of Type 1 diabetes. We studied 26 children (14 males and 12 females) aged 1.4–16.2 years, with recently-diagnosed Type 1 diabetes followed-up for 2–5 years. Nine patients had been breast-fed and 17 bottle-fed. In all patients we evaluated IgA and IgG antibodies to beta-lactoglobulin by ELISA. The control group comprised 59 healthy age- and sex-matched subjects. At diagnosis, 13 patients (50%) had elevated beta-lactoglobulin IgA (> +2SD) and 2 (7.7%) elevated beta-lactoglobulin IgG antibodies (> +2SD). ICA were present in 19 of 26 (73%) and anti-insulin antibodies in 6 of 26 (23%) patients.

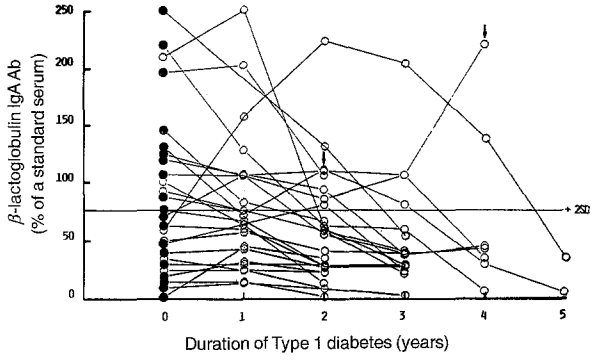


**Fig. 1.** Beta-lactoglobulin IgA antibody (IgA-ab) levels in 26 children with Type 1 diabetes at diagnosis and during 2–5-year follow-up. ● ICA-positive patients; ○ ICA-negative patients; ↓ the two patients with elevated beta-lactoglobulin IgA-antibody levels at the end of the follow-up. The line at 75% indicates 2SD

Over the 2–5-year follow-up we observed a transient increase in beta-lactoglobulin IgA antibodies in two other patients. At the end of follow-up beta-lactoglobulin IgA antibodies decreased to normal levels in all but two females (Fig. 1) and beta-lactoglobulin IgG antibodies in all patients. No correlation was found between beta-lactoglobulin IgA or IgG antibodies and age, breast-feeding duration, islet-cell, anti-insulin or other organ and non-organ specific antibodies and HLA types. No difference was found in beta-lactoglobulin antibody levels between breast- or bottle-fed patients.

Some investigators have suggested that breast-feeding may protect against the risk of Type 1 diabetes in later life [2–4], but the results of more recent studies are conflicting [5]. It has been hypothesized that intestinal permeability is increased in Type 1 diabetes [6]. On the other hand a strong antigenic similarity has been observed between human beta-casein and bovine beta-lactoglobulin [7] and the presence in human milk of beta-lactoglobulin from cow's milk in the mother's diet has been confirmed [8]. These observations together with our findings that anti-beta-lactoglobulin antibodies tend to disappear during follow-up, suggest that they represent only a transient abnormal immunological response. These antibodies may reflect increased production of antibodies against different antigens by B-lymphocyte clones [9].

Yours sincerely,
R. Lorini, M. A. Avanzini and L. Vitali

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# Response from the authors

**Anti beta-lactoglobulin antibodies in children with Type 1 (insulin-dependent) diabetes mellitus – what do they mean?**

Dear Sir,

Prof. Lorini and associates report an interesting follow-up study of 26 children with Type 1 (insulin-dependent) diabetes mellitus followed-up for 2–5 years for beta-lactoglobulin IgA and IgG antibodies and also islet cell and anti-insulin antibodies. The initially increased values of beta-lactoglobulin antibodies in Type 1 diabetic patients compared to control subjects tended to decrease over time. It is concluded that the increased antibodies represent only a transient abnormal immunological response and therefore have no association with the triggering of autoimmunity. Part of the authors results could be explained by the statistical phenomenon called regression to the mean. A slow decrease in antibody titre by time may, however, be compatible with the theory that cow's milk protein antigens, when introduced early in life, may be causally related to the initiation of autoimmunity. The authors did not find any correlation between beta-lactoglobulin antibody levels and breast feeding duration or bottle feeding in contrast to our recent study [1]. This lack of significance might well be a type 2 error because of the very small number of patients studied. Possibly for the same reasons, these authors found no correlation between beta-lactoglobulin antibodies and islet cell antibodies as was reported in our study [1]. Islet cell antibodies that react with cytoplasmatic antigens are regarded by many authors today as an epiphenomenon reflecting an ongoing beta-cell destruction. A strong correlation between cow's milk antibodies and islet cell antibodies would indeed indicate a non-specific polyclonal activation leading to an increase in beta-lactoglobulin and other antibodies parallel to the islet cell antibody increase. In our study, however, in a multiple regression analysis we found increased levels of beta-lactoglobulin antibodies to be associated with a significant increase in risk for Type 1 diabetes also when islet cell antibodies were standardised for, which could imply an independent and thus a more specific effect. Several epidemiological studies have clearly indicated an association between risk of Type 1 diabetes and a short duration of breast feeding and/or an early introduction of formula feeding [2–8]. During the first months of life the human intestine is leaky to large molecules which is therefore reason to believe that some cow's milk proteins may hide one (of the probably several) triggers of autoimmunity in Type 1 diabetes. Recently, interesting data have been presented [9] showing that bovine albumin rather than beta-lactoglobulin in cow's milk might be the trigger since it shares an amino acid sequence (ABBOS) with certain DR- and DQ-class II MHC proteins and that antibodies against this bovine serum albumin region could crossreact with a beta-cell membrane protein. It was also shown that recent-onset diabetic children had increased levels of not only anti-bovine albumin antibodies but also anti-ABBOS antibodies compared to healthy control subjects. It was therefore suggested that if the ABBOS-peptide was supplied early in life it could induce an immune reaction that could be boostered even after gut maturation by the beta-cell surface protein sharing an epitope with ABBOS. This kind of mechanism is of course compatible with the findings of increased levels of antibodies at onset of disease subsequently decreasing parallel to the loss of beta cells.

It is certainly not clear whether, and in that case which, cow's milk proteins may be involved in the aetiology of Type 1 diabetes. The ABBOS hypothesis is, however, so far the best substantiated and since probably beta-lactoglobulin antibodies and other cow's milk proteins antibodies will be increased parallel to an increase of ABBOS-antibodies the findings of many groups including the interesting report from Prof. Lorini's group may fit together. Clearly more research is needed to verify the cow's milk protein theory but it is so far one of the most promising as to primary prevention possibilities for Type 1 diabetes.

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